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Electrochemical behavior of kaempferol and its determination in presence of quercetin employing multi-walled carbon nanotube modified carbon paste electrode

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ABSTRACT

The electrochemical behavior of kaempferol was investigated by cyclic voltammetry (CV) and square wave voltammetry (SWV) at plane and multiwalled carbon nanotubes modified carbon paste electrode (MWCNTs/CPE). Kaempferol produces an anodic peak at MWCNTs modified CPE with a quasi-reversible nature in phosphate buffer of pH 7.73. The oxidized species of kaempferol was found to be stable ($Ip^a/Ip^c \approx 1$) over scan rates of 100–600 mV^{-1}. The same electrode was also found to catalyze the electrode oxidation of quercetin in presence of kaempferol under similar conditions which enabled the simultaneous determination of kaempferol and quercetin. Linearity of peak currents (Ip) vs. concentrations of kaempferol and quercetin was found in the range of 6.72×10^{-9} M to 40.34×10^{-9} M with a detection limit of 2.90×10^{-9} M for kaempferol and 13.0×10^{-9} M to 50.9×10^{-9} M with a detection limit of 3.5×10^{-9} M for quercetin using fast and sensitive SWV. The developed method has been applied for the quantitative analysis of kaempferol in corms and petals of Indian traditional medicine saffron (*Crocus sativus*) using a phosphate buffer of pH 7.73 and the average percent recoveries obtained are 99.84 and 99.26, respectively.

1. Introduction

Kaempferol is a flavonol and phytonutrient which has been isolated from green tea [1], grapefruit, apples, saffron [2–4] and other plant sources. Kaempferol is a yellow crystalline solid with a melting point of 276–278 °C. It is slightly soluble in water but soluble in hot ethanol and ether. Many glycosides of kaempferol, such as kaempferitrin and astragalin, have been isolated as natural products from plants. Kaempferol consumption in tea and broccoli has been associated with reduced risk of heart disease. The compound has antidepressant properties [5,6]. Kaempferol is well known for its cancer fighting properties and has been linked with the prevention of breast cancer, ovarian cancer and prostate cancer. Kaempferol consumption appears to reduce the risk of pancreatic and lung cancer also [7]. It has also been shown to prevent arteriosclerosis (hardening and loss of elasticity within the arteries) and heart disease. Additionally, kaempferol is a powerful antioxidant and phytoestrogen (negative menopausal symptoms). Kaempferol has also been linked with increasing metabolism by supporting the production of the metabolism boosting hormone triiodothyronine (also known as T3). Quercetin is also a flavonoid having an analogous structure of kaempferol. It is found in many fruits, vegetables, leaves and grains. It can be used as an ingredient in supplements, beverages, or foods. It is contraindicated with some antibiotics; and interacts with fluoroquinolones (an antibiotic), as quercetin competitively binds to bacterial DNA gyrase [8]. Therefore, determination of kaempferol and quercetin becomes significant.

Electrochemical assay often offers selectivity and sensitivity due to the selective detection of electroactive species among the complex samples. The electrochemical oxidation of a number of natural phenolics using cyclic voltammetry has been reported in the literature [9]. However, there is no report for the simultaneous determination of kaempferol and quercetin employing any electrochemical method.

Chemically modified electrodes (CMEs) show certain merits like easy surface renewal, low cost, low residual current and ease of

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fabrication. Hence, CMEs have been successfully utilized for determination of various drug molecules in our laboratory [10–13]. Carbon nanotubes (CNTs) have become the subject of intense researches in the last decades because of their unique properties and the promising applications in any aspect of nanotechnology. CNTs are widely used in electronic, biomedical, pharmaceutical, catalytic, analytical, and material fields. Particularly, the properties of small dimensions, functional surfaces, good conductivity, excellent biocompatibility, modifiable sidewall, high reactivity, the large specific area producing high sensitivity, tubular nanostructure and the chemical stability make CNTs ideal candidates for constructing sensors with high performances. The electron transfer and the direct electrochemistry of redox proteins at CNT-based electrochemical sensors were also widely reported. Due to the well-defined structure and the electrocatalytic activity towards many substances, CNTs are also extensively used as the carrier platforms for constructing various electrochemical sensors. The promising applications of CNTs have been reviewed by several authors [14–22].

Chemical structure of kaempferol as well as quercetin (Fig. 1) contains flavonoid ring which contains electrochemically oxidisible groups in the flavone moiety which prompted us to study its electrochemical behavior at multi-walled carbon nanotube modified carbon paste electrode using cyclic voltammetry (CV) and square wave voltammetry (SWV) for developing a fast and sensitive square wave voltammetric (SWV) procedure for their determination. Various voltammetric [23–25] and chromatographic methods [26–31] for the determination of kaempferol in different biological samples have been reported in the literature. However, there is no report for its determination at MWCNT-modified CPE, particularly in presence of quercetin.

2. Experimental

2.1. Reagents and materials

Pure kaempferol, quercetin and carbon nanotube powder (Sigma grade) were used. Other chemicals used in the present work were either of Anala R or Himedia Laboratories Pvt. Ltd. Mumbai, grade. Standard stock solution each of kaempferol and quercetin were prepared by dissolving its 0.1 mg in 100 ml of pure ethanol and was stored in the dark. The supporting electrolyte, 0.1 M phosphate buffer solutions of different pH and ionic strength were prepared by mixing standard solutions of Na_2HPO4 and NaH_2PO_4. Saffron corms and petals were obtained from the fields of saffron in Pampur, Srinagar, Kashmir (J&K), India.

2.2. Instrumentation

All voltammetric experiments were performed with Ω Metrohm 797 VA Computrace Potentiostat (Switzerland) through electrochemical software version 3.1. A three-electrode cell was employed incorporating a hand-made working MWCNT electrode, an Ag/AgCl (3.0 M KCl) reference electrode and a platinum wire counter electrode. Mass transport was achieved with a teflon-coated bar at approximately 400 rpm using a magnetic stirrer (KIKA Labortechinik, Germany). A systronics digital μpH meter model-361 was used for pH measurements. All experiments were performed at room temperature 25 ± 2 °C and dissolved oxygen was removed by passing pure nitrogen through the solutions.

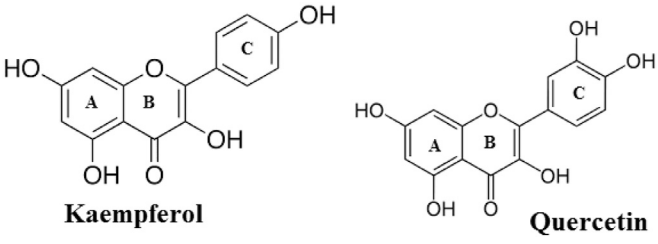


Fig. 1. Structure of kaempferol and Quercetin.

Carbon nanotube electrode was prepared in usual way by hand-mixing graphite powder (Aldrich; 1–2 mm), carbon nanotube powder (Sigma) and mineral oil (Sigma). The ratio of these three was 60:10:30. The prepared paste was filled into the teflon well. A copper wire fixed to a graphite rod and inserted in to the teflon well serves to establish electrical contact with the external circuit. A good reproducibility of electrode response was achieved by simply renewing the surface of paste electrode. New electrode surface was formed by mechanically pressing the paste from the top of the teflon well and smoothening of the electrode surface was done by rolling a smooth glass rod on the electrode surface and finally it was cleaned carefully by distilled water. Each measurement involved a fresh carbon nanotube surface.

2.3. General analytical procedure

Stock solution of kaempferol/quercetin was prepared by dissolving its required amount in ethanol. A 25 ml aqueous solution of analyte containing 5 ml of 0.1 M phosphate buffer/5% (v/v) ethanol pH 7.73 and a specific amount of sample solution was added to the cell and purged with purified nitrogen for 5 min to remove oxygen. Voltammograms were recorded in exploratory mode. The scanning potential was varied from 0 to +2.0 V. The stirring was then stopped and after a delay period of 10 s to settle the solution and decrease the background current, cyclic voltammogram was recorded by the anodic potential scanning. Then electrode was first activated in 0.02 M phosphate buffer/ethanol pH 7.73 using successive cyclic sweep from 0 V to 2.0 V until the cyclic voltammograms were stable. After that, a known concentration of drug was added into the solution and the voltammogramms were recorded.

2.4. Isolation of kaempferol from saffron petals

Powdered petals of *Crocus sativus* (10 g) were extracted with methanol containing 1% concentrated HCl at room temperature, until complete discoloration of petals. The combined methanol extract was evaporated in vacuum at 30 °C to leave a crude extract (0.5 g). The crude extract was hydrolyzed with HCl (1.8 N, 5 ml for each 15 mg crude extract) under nitrogen atmosphere according to the literature [2,32]. The resulting solution was extracted with amyl alcohol (5 ml × 3). The organic layer was washed with water until the pH of the aqueous phase remained constant. Organic phase was evaporated in vacuum to leave a residue. The residue was used in analysis.

2.5. Isolation of kaempferol from saffron corms

Grinded pieces of roots (corm) of *C. sativus* were extracted with ethanol. Extraction with stirring was done for 48 h. Extract was filtered and concentrated. Thin layer chromatography of extract with (1:9 chloroform methanol) showed three pink, red, and yellow colored spots. All the three spots were compared with authentic kaempferol and yellow one matched with kaempferol. It was separated by column containing silica gel using 1: 9 (chloroform: methanol) solvent system. Three coloured fractions were collected. The solvent of yellow fraction was evaporated in vacuum to leave a yellow powdered residue which was stored and used in analysis.

3. Interday and intraday assay

Blank samples spiked with standard concentrations were used in the evaluation of interday and intraday assays. In this experiment, two standards were spiked to an unknown sample. Each spiked sample was determined in triplicate for three consecutive days. The interday and intraday precisions were evaluated using the relative standard deviation. Also, the same electrode was used for three consecutive days for evaluating the stability of the electrode.

4. Results and discussion

4.1. Effect of modifier

Fig. 2A shows the effect of MWCNTs on the electrode oxidation of kaempferol in 0.02 M phosphate buffer (pH = 7.73) using cyclic voltammetry (CV). It is observed that kaempferol produces a weak quasi-reversible couple at plane CPE (Fig. 2A, b). However, at the same concentration and scan rate both cathodic and anodic peak currents increase significantly with a negative shift in both cathodic and anodic peak potentials at MWCNTs modified CPE (Fig. 2A (c). Fig. 2A(a) is the CV curve of blank containing 0.02 M phosphate buffer 5% (v/v) ethanol (pH = 7.73) at MWCNT modified CPE. Further, differential pulse voltammetry (DPV) was also employed to study the effect of modifier. Kaempferol shows a weak oxidation peak at plane CPE (Fig. 2B, a) and at the same concentration and scan rate, the oxidation peak current of kaempferol enhances at MWCNTs modified CPE with a negative shift in peak potential (Fig. 2B, b). Therefore, an increase in peak current of kaempferol and a negative shift of peak potential in CV as well as DPV shows that MWCNTs have catalytic effect on electrode oxidation of kaempferol. This can be undoubtly attributed to the unique structure and properties of MWCNT (such as very large specific area producing high sensitivity, good conductivity, modifiable sidewall, tubular nanostructure). Effect quantity of MWCNTs along with carbon paste and mineral oil was also studied using different electrodes containing 0.1–1.0% MWCNTs. The peak current increases upto 0.5% and then remains constant. Hence, 0.5% of MWCNTs was employed in each experimental study. In short, 0.5% MWCNT-modified CPE greatly improves the determination sensitivity of kaempferol.

4.2. Effect of scan rate and pH of supporting electrolyte

Fig. 3A shows the cyclic voltammograms of kaempferol in the phosphate buffer of pH 7.73 at different scan rates with well-defined anodic and cathodic waves produced at the MWCNT-modified CPE surface. The plot of anodic peak current vs. square root of scan rate (Fig. 3B) gives a linear plot, pointing to a diffusion controlled response [33]. Its electrochemical behavior is quasi-reversible in the phosphate buffer of pH 7.73. The cyclic voltammetric data of kaempferol in pH 7.73 and 6.87 media are listed in Table 1A and 1B. The oxidized species of kaempferol in the phosphate buffer of pH 7.73 was stable by showing the ratio of Ip^c/Ip^a of approximately at one over the scan rates of 100~600 mV^{-1}, while the ratio of Ip^c/Ip^a was 0.9 at pH 6.87. As the pH values of supporting electrolytes were increased, the oxidized species of kaempferol becomes more stable as indicated by the increasing ratio of Ip^c/Ip^a shown in Fig. 3C and Table 2A. When the E_{pa} was plotted against pH values, slope was −55 mV/pH with a correlation coefficient of 0.968 (Fig. 3D). The plot of E_{pc} against pH values yielded the slope of −57.5 mV/pH with a correlation coefficient of 0.907. From this study, it is confirmed that protons were involved in the electrochemical oxidation of kaempferol and the ratio of H^+/e^- was one.

The square wave voltammograms of kaempferol also showed the well-defined single anodic peak which shifted towards negative (less positive potentials) as the pH values of supporting electrolytes were increased as shown in Fig. 4A. The plot of peak potentials (Ep) of kaempferol vs. pH values of the supporting electrolytes resulted in linearity with a slope of – 54.0 $mVpH^{1-}$ (Fig. 4B) confirming the proposed oxidation mechanism. The peak currents (Ip) of 4.34×10^{-6} M kaempferol in the different pH values of phosphate buffers were as follows: the average Ip ± standard deviation for triplicate measurements was 9.21 ± 0.13 μA in pH 7.73 buffer, 8.71 ± 0.05 μA in pH 6.87 buffer, 7.71 ± 0.03 μA in pH 6.47 buffer, 7.01 ± 0.05 μA in pH 5.91 buffer and 6.75 ± 0.05 μA in pH 4.53, respectively. Since the most sensitive peak current (I_p) of kaempferol was obtained in pH 7.73 medium, the further analytical works have been studied by square wave voltammetry using a pH 7.73 phosphate buffer.

4.3. Electrode reaction mechanism of kaempferol

Cyclic and square wave voltammetric study has shown that kaempferol undergoes electrochemical oxidation in phosphate buffer/5% (v/v) ethanol at pH 7.73. The electrode process is quasi-reversible at MWCNT-modified CPE and involves equal number of electrons and protons. The value of 'n' number of electrons involved in the electrode reaction was calculated using the equation; ΔE = 0.059/n, where 'n' is number of electrons involved in the

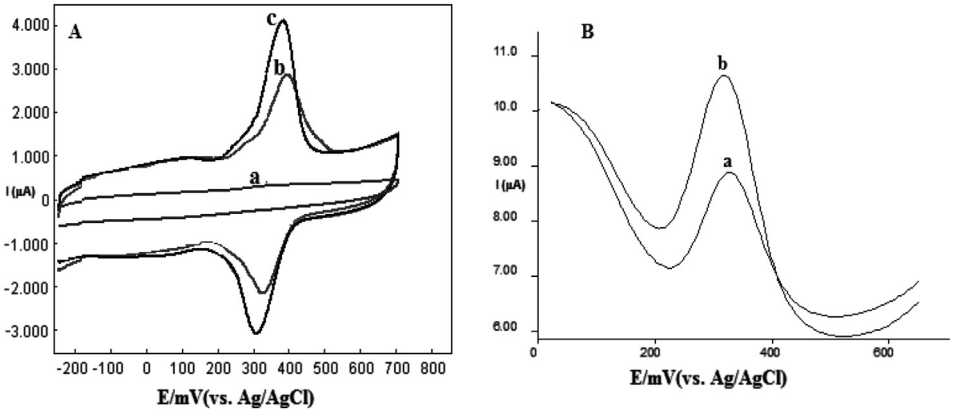


Fig. 2. **A** (**a**) Cyclic voltammograms of blank containing 0.02 M phosphate buffer/5% (v/v) ethanol (pH = 7.73), (**b**) in presence of 6.72×10^{-6} M kaempferol at plane CPE and (c) at MWCNT-modified CPE at a scan rate of 100 mV s^{-1}. (B) Differential pulse voltammograms of 6.72×10^{-6} M kaempferol at (a) plane CPE and (b) at MWCNT-modified CPE at a scan rate of 100 mV s^{-1}.

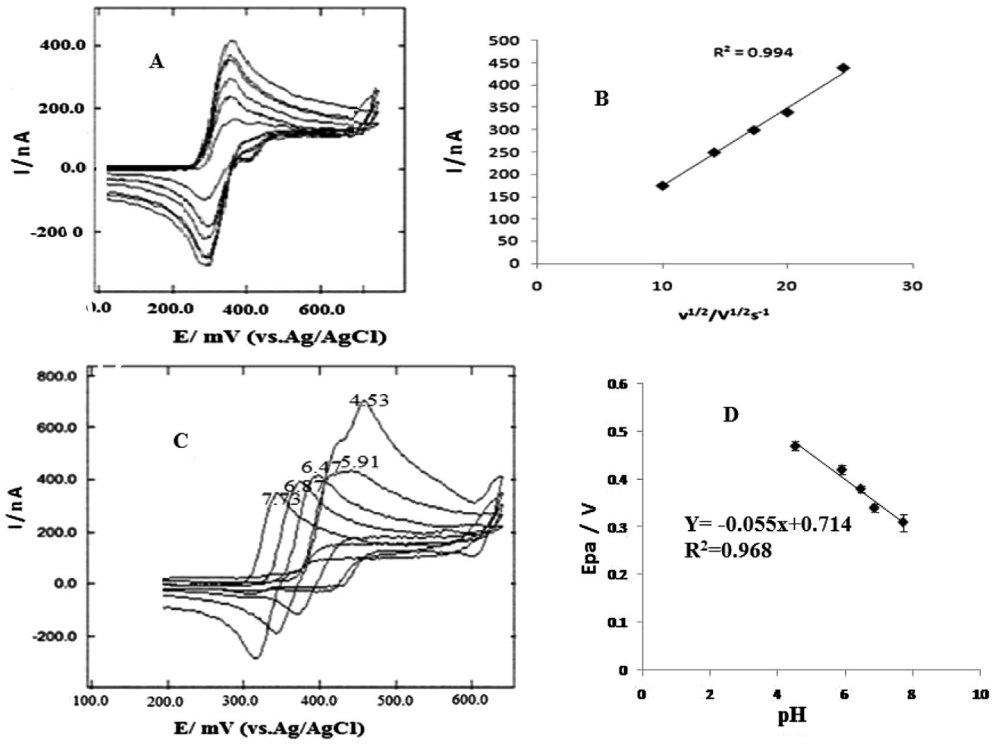


Fig. 3. (**A**) Cyclic voltammograms of 6.72×10^{-6} μM kaempferol in phosphate buffer, pH 7.73 at MWCNT-modified CPE. Scan rates: 100 mV s^{-1}, 200 mV s^{-1}, 300 mV s^{-1}, 400 mV s^{-1}, 500 mV s^{-1}, and 600 mV s^{-1}. (B) Linear plot of anodic peak currents versus square root of scan rate. (C). The cyclic voltammograms of 6.72×10^{-6} M kaempferol in the phosphate buffer of different pH values. (From right to left: 4.53, 5.91, 6.47, 6.87 and 7.73) at MWCNT-modified CPE at a scan rate of 100 mV^{-1}. (D) The plot of peak potentials (Ep_a) of kaempferol in CV mode vs. pH values of the supporting electrolytes. Data in the plot represent the mean ± S. D (n = 3).

Table 1A
Cyclic voltammetric data of 6.72×10^{-6} M kaempferol in phosphate buffer of pH 7.73 with different scan rates.

Scan rate (mV)	Anodic		Cathodic		ΔE_p(mV)	Ip^c/Ip^a
	Ep^a(V)	Ip^a(nA)	Ep^c (V)	Ip^c (nA)		
100	0.326	165.50	0.299	215.50	27	1.302
200	0.320	233.10	0.297	286.66	23	1.229
300	0.318	307.10	0.291	341.50	27	1.112
400	0.320	306.90	0.290	396.10	30	1.290
500	0.322	355.20	0.295	400.10	27	1.126
600	0.335	402.00	0.310	433.20	25	1.077

Table 1B
Cyclic voltammetric data of 6.72×10^{-6} M kaempferol in a pH 6.87 phosphate buffer with different scan rates.

Scan rate (mV)	Anodic		Cathodic		ΔE_p(mV)	Ip^c/Ip^a
	Ep^a(V)	Ip^a(nA)	Ep^c(V)	Ip^c(nA)		
100	0.350	162.50	0.297	138.12	33	0.850
200	0.335	223.10	0.290	191.19	35	0.857
300	0.329	301.10	0.291	267.97	28	0.890
400	0.327	310.90	0.287	278.77	36	0.899
500	0.332	339.20	0.324	324.61	28	0.957
600	0.339	398.10	0.302	382.17	37	0.960

reaction, ΔE is the separation between forward and reverse peak potentials. In the present case, the value of ΔE is found to be 26 mV which corresponds to two electron transfer reaction.

This study is also supported using controlled potential coulometry, the number of electrons transferred, n, were calculated from the charge consumed by the desired concentration of kaempferol. The charge consumed was determined at pH 7.73. For

Table 2A
Cyclic voltammetric data of 6.72×10^{-6} M kaempferol in the phosphate buffers of different pH values. (Scan rate = 500 mV s^{-1}).

pH	Anodic		Cathodic		ΔE_p(mV)	Ip^c/Ip^a
	Ep^a(V)	Ip^a(nA)	Ep^c(V)	Ip^c(nA)		
4.53	0.470	670.39	0.421	128.49	28	0.191
5.91	0.430	428.57	0.394	178.57	31	0.416
6.47	0.380	424.00	0.363	288.00	26	0.679
6.87	0.320	374.74	0.322	353.74	33	0.943
7.73	0.310	306.93	0.314	396.04	35	1.290

this purpose 4.6×10^{-6} M solution of kaempferol was placed in the cell and electrolysis was carried out at +0.45 V against Ag/AgCl reference electrode. During the electrolysis, solutions were continuously stirred and purged with nitrogen. Number of electrons 'n' was calculated using the equation Q = nFN, were Q is charge in coulombs, F is Faraday's constant and N is number of moles of the substance. Three experiments were performed and the value of 'n' was found to be 2.01, 2.05 and 2.11, thus indicating that the electrode reaction is a two electron process. Therefore, the probable oxidation mechanism corresponds to the hydroxyl groups in the rings C and B of kaempferol and the following mechanism is proposed in Scheme 1.

4.4. Optimization of operational parameters

Variation of peak current of kaempferol at MWCNT-modified CPE was investigated using square wave and differential pulse voltammetric modes. Both the techniques gave comparable results. But square wave has been chosen for optimizing the operational parameters. The important instrumental variables such as pulse

Table 2B
Analytical parameters for voltammetric determination of kaempferol and quercetin using SWV

Parameter	Kaempferol	Quercetin
Measured potential(V)	0.38	0.29
Linear range(M)	6.72×10^{-9} to 40.34×10^{-9}	15.0×10^{-9} to 56.9×10^{-9}
Slope($\mu A/\mu gmL^{-1}$)	1.004	1.461
Correlation coefficient(r^2)	0.970	0.970
S_a	0.005	0.05
LOD(μM)	2.90×10^{-9}	3.59×10^{-9}

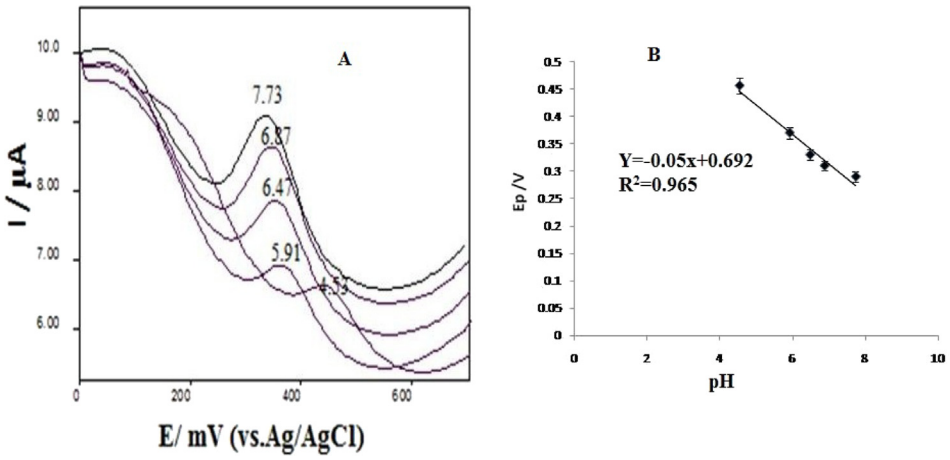
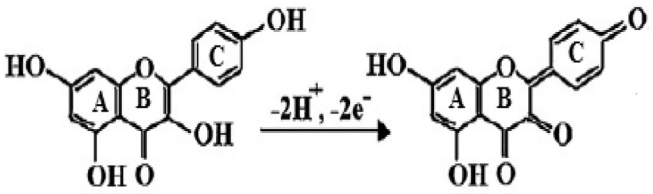


Fig. 4. (**A**) The square wave voltammograms of 6.72×10^{-6} M kaempferol in the phosphate buffer of different pH values. From right to left: pH 4.53, pH 5.91, pH 6.47, pH 6.87, 7.73 Working electrode: MWCNT-modified CPE, Equilibration: 20 s, Frequency: 120 Hz, Pulse height: 25 mV. Scan increment: 2 mV, scan rate = 100 mV s^{-1}. (**B**) The plot of peak potentials (Ep) of kaempferol in squarewave mode vs. pH values of the supporting electrolytes. Data in the plot represent the mean ± S. D (n = 3).



Scheme 1. Probable oxidation mechanism corresponds to the hydroxyl groups in the rings C and B of kaempferol and the following mechanism is proposed.

amplitude (ΔE_{sw}) scan increment (Δs) and frequency (f) were examined. Frequency was varied from 10 to 150 Hz using a scan increment of 100 mV; pulse amplitude of 50 mV. Peak current was maximum at 50 Hz which was chosen for entire analysis. The effect of scan increment on peak current of the drug at MWCNT-modified CPE at pH 7.73 revealed that the peak current increases upon the increase of scan increment (10–100). A scan increment of 100 mV was used in the present study. At pulse amplitude of 50 mV, the peak current was found to be much more sharp and defined.

4.5. The calibration curve

The peak currents (Ip) of kaempferol was found to increase linearly with the concentration from 6.72×10^{-9} M to 40.34×10^{-9} M in the phosphate buffer of pH 7.73 using SWV and the calibration curve is shown in Fig. 5A. For triplicate measurements at each concentration, the relative standard deviation ranged from 0.2% to 4% (Fig. 5B). The peak potentials (Ep) at these concentrations appeared at 0.354 ± 0.007 V. However, the Ip value was less than expected from the calibration curve at concentrations higher than 40.34×10^{-9} M. At concentrations lower than 6.72×10^{-9} M, the Ip was not significantly varied against concentration.

4.6. Interference study and simultaneous determination of kaempferol and quercetin

For the possible analytical application of the proposed method, the effect of some common excipients used in pharmaceutical preparations were studied by analyzing sample solutions containing a fixed concentration of kaempferol (6.72×10^{-6} M) spiked with varying concentrations of each excipient under the similar experimental conditions. Some tested excipients, such as glucose, sucrose and lactose, were used. The interference studies were done by dissolving these excipients in ethanol/aqueous solution. However, no interference study has been done with starch and gelatin because they are not soluble in alcohol/aqueous medium and hence could not interfere. The determination of kaempferol in the presence of above mentioned excipients was evaluated and a recovery ranging from 97.18 to 98.59% was obtained. The results show that no serious interference occurred from the classical additives tested. This shows a good specificity of the method in presence of these substances.

However, effect of quercetin was elaborately studied as it produces a separate oxidation peak. Fig. 6 shows the effect of modifier (MWCNTs) on electrode oxidation of kaempferol and quercetin simultaneously using SWV at a concentration of 3.44×10^{-6} M. It is observed that kaempferol and quercetin give a separate peak with an enhancement in the peak current of both kaempferol and quercetin at MWCNTs - modified CPE (Fig. 6b) as compared to plane CPE (Fig. 6a). This shows that MWCNTs catalyze the electrode oxidation of kaempferol as well as quercetin. This concept was applied for the simultaneous determination of kaempferol and quercetin. Simultaneous determination of kaempferol and

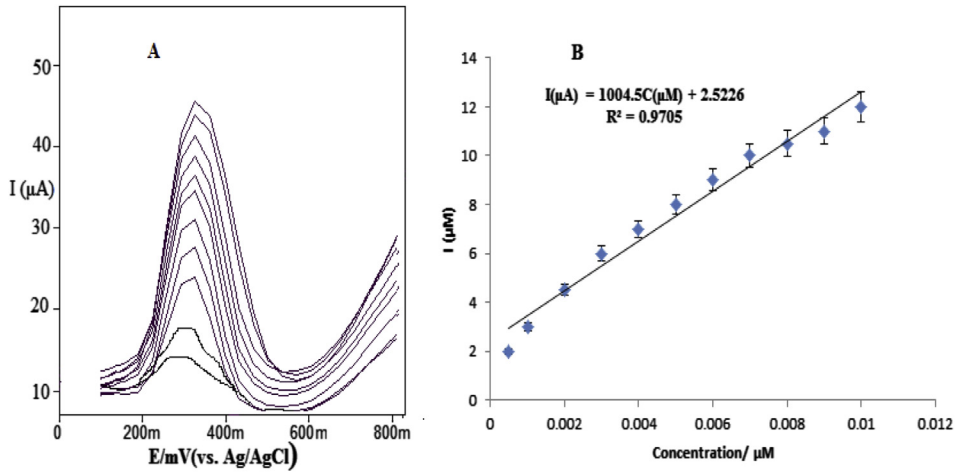


Fig. 5. (**A**) The dependence of the square wave current for kaempferol at different concentrations in the phosphate buffer; pH 7.73, frequency (f) = 100 Hz, pulse amplitude = 50 mV, scan increment = 10 mV, Scan rate: 100 mV s^{-1}. (**B**) Standard calibration plot of concentration vs. current. Data represent the mean ± S. D (n = 3). I(μA) = 1004.5C (M)+2.522,R^2 = 0.970.

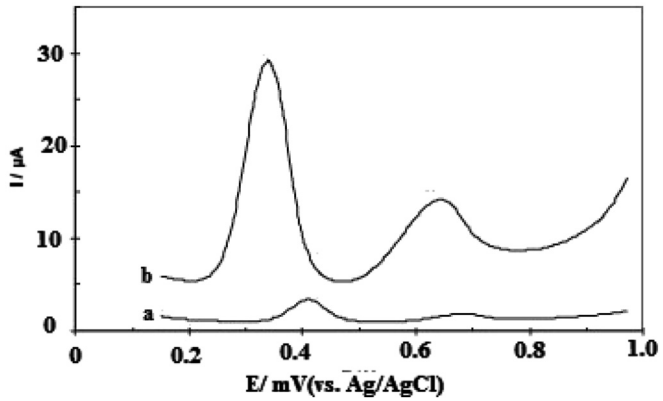


Fig. 6. Square wave voltammograms of 13.44 × 10^{-6} M kaempferol and quercetin at (a) plane CPE and (b) MWCNTs modified CPE in 0.02 M phosphate buffer/5% (v/v) ethanol (pH = 7.73) at a scan rate of 100 mV s^{-1}.

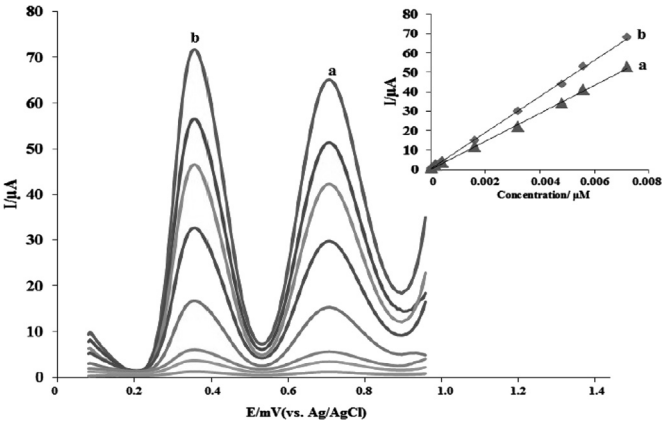


Fig. 7. Square wave voltammograms of kaempferol and quercetin at varying concentration of each in phosphate buffer; pH 7.73, frequency (f) = 100 Hz, pulse amplitude = 50 mV, scan increment = 10 mV, Scan rate: 100 mV s^{-1}. Insert is the calibration plot of concentration vs. current of kaempferol and quercetin.

quercetin with varying concentrations of each at the MWCNTs modified CPE using SWV (Fig. 7). Two well-defined peaks are observed for kaempferol and quercetin at 0.32 V and 0.71 V, respectively. It is observed from the figure that there is no interference effect up to 40.34 × 10^{-9} M, thus, allowing out the method to simultaneously detect and quantify kaempferol and quercetin. The calibration plot was obtained from the SWV analysis for kaempferol and quercetin (insert in Fig. 7).

4.7. Validation of the proposed method

The applicability of the proposed square wave voltammetric (SWV) method as analytical method for the determination of kaempferol and quercetin was examined by measuring the peak current as a function of concentration of the drug for at least three times under the optimized operational parameters. The calibration plot of the peak current versus the concentration was found to be linear over a wide range for both the molecules and linear regression equations are expressed as:

$$\text{Kaempferol}: [i_p\ (\mu A) = (1.004)\ \text{concentration}\left(\mu g m L^{-1}\right) + 2.45], \ r^2 = 0.990$$

$$\text{Quercetin}: [i_p(\mu A) = (1.461)\ \text{concentration}\left(\mu g m L^{-1}\right) + 3.32], \ r^2 = 0.980$$

The regression plots showed that there is a linear dependence of the current intensity on the concentration for both kaempferol and quercetin over the range as given in Table 2B. The table also shows the detection limits and the results of the statistical analysis of the experimental data such as slope, intercept, the correlation coefficients obtained by the linear least squares treatment of the results along with standard deviation (S. D.) of intercept (S_a) on the ordinate. The good linearity of the calibration graphs and the negligible scatter of the experimental points are clearly evident by the values of correlation coefficient and S.D. The limit of detection was calculated as; LOD = 3Sa/k, where S_a is the standard deviation of intercept and k is the slope of regression line [34,35]. The calculated detection limit for the standard solution for kaempferol was 2.90 × 10^{-9} M and for quercetin was 3.59 × 10^{-9} M. The peak is not resolved from the noise at concentrations lower than the LODs.

Table 3A
Precision and accuracy for assay of kaempferol and quercetin in preanalyzed samples by the proposed SWV procedure.

Kaempferol					Quercetin			
Added (nM)	Found[a] (nM)	%R)	Precision (%R.S.D)	Accuracy (%Bias)	Found[a] (nM)	%R	Precision (%R.S.D)	Accuracy (%Bias)
6.99	7.06	100.50	1.80	0.48	6.95	99.50	1.52	−0.48
13.98	13.91	99.25	0.90	−0.74	14.37	101.5	1.12	1.43
27.97	28.67	100.75	1.02	0.23	27.90	99.75	0.98	−0.24

('a' represent the average of five replicate measurements).

Table 3B
Comparison of the detection limit of the proposed method for kaempferol with the other reported methods

Method	Detection limit	Reference no.
HPLC with diode array detector	2.76–10.13 nM	[24]
HPLC with chemiluminescence detection	3.4 nM	[25]
HPLC with UV detector	3.8 nM	[27]
Voltammetry(conventional)	0.10 μM	[21]
Square wave voltammetry	2.90 nM	Present work

4.8. Accuracy, precision and reproducibility

The accuracy of the developed method was carried out by spiking with accurately preanalyzed amounts of kaempferol and quercetin. The accuracy is expressed as a mean relative error (measured concentration – preanalyzed concentration/preanalyzed concentration × 100). The mean percent recoveries are 101.166 and 101.25 for kaempferol and quercetin, respectively and the values of mean relative error are acceptable (Table 3A), this shows the best accuracy obtained by using these methods.

The precision and reproducibility of the developed method (SWV) for kaempferol and quercetin were determined in three replicate analyses (Table 3A). The precision of the proposed procedure was estimated by analyzing kaempferol and quercetin in preanalyzed assay solutions for three times in four successive days using SWV. The percentage recoveries based on the average of five separate determinations are given in Table 3A. The results confirmed both the good precision of the proposed procedure and stability of the drug's solution. The mean variation coefficients are 1.24 and 1.20% for kaempferol and quercetin, respectively. The variation coefficients are less than 2.0% indicating that the method is precise and confidence.

5. Comparison of the sensitivity of the method with previously reported methods

Table 3B compares the detection limit of the proposed method with the other reported methods [25,28,29,31]. It is obvious that the sensitivity of the method is superior to all previously reported methods. The data in the table reveal that the detection limit of the method is lower than all previous reported methods.

6. Analytical applications

The optimized sensitive SWV procedure was successfully applied for the determination of kaempferol in a well known Indian traditional medicine (i.e. corms and petals of saffron). Prior to determination kaempferol was isolated separately from corms as well as petals of this plant as discussed above. The isolated kaempferol was dissolved in ethanol and was diluted with supporting electrolyte before determination. Determination of kaempferol was performed by square-wave voltammetry using standard addition calibration method (Fig. 8A and B). The results are shown in Table 4. The content of kaempferol in saffron is

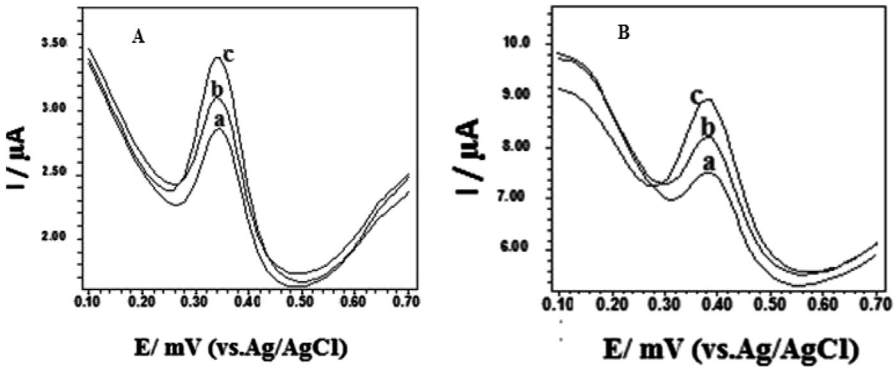


Fig. 8. Square wave voltammetric determination of kaempferol by standard addition calibration in the phosphate buffer at pH 7.73. Scan rate: 100 mV s^{-1} in (**A**) Saffron corms, (**B**) Saffron petals. In each case 'a' represent the concentration of unknown sample and 'b' and 'c' represent the standard additions first and second respectively.

Table 4
Measurement results of kaempferol in saffron corms and petals using SWV in the phosphate buffer; pH 7.73. Scan rate: 100 mV s^{-1}.

Sample	Saffron corms				Saffron petals			
S.No.	Added nM	Expected nM	Found nM	Recovery %	Added nM	Expected nM	Found nM	Recovery %
0	–	13.42	–	–	–	13.63	–	–
1	8.74	22.16	22.20	100.157	8.74	22.37	22.06	98.593
2	17.48	30.90	30.83	99.730	17.48	31.11	31.18	100.224
3	22.2	39.65	39.51	99.647	26.22	39.86	39.47	99.035

calculated and it is 2.73 ± 0.015 μg/g of dry weight of corms and 23.5 mg/g of dry weight of petals which is in accordance with that reported in literature [2–4]. The relative standard deviation was 2.03% and 1.94% (n = 5) for kaempferol determination in corms and petals respectively. In addition, some recovery experiments were carried out and the recovery varied from 99.25 to 100.75 and 98.593 to 100.224 in corms and petals respectively. The relative standard deviation for intraday and interday assay was 0.89 and 0.73% (n = 3) respectively.

7. Conclusions

Electrochemical behavior of kaempferol at MWCNTs modified CPE is first time being reported here. Cyclic voltammetry has shown that kaempferol gives a quasi-reversible redox couple at MWCNTs modified CPE in phosphate buffer of pH 7.73 with two electron and two proton transfer. The oxidized species of kaempferol in the phosphate buffer of pH 7.73 was stable by showing the ratio of Ip^c/Ip^a of approximately one over the scan rate of 100~600 mV s^{-1}, while the ratio of Ip^c/Ip^a was 0.9 in the supporting electrolytes of pH 6.87 which shows the oxidized species is more stable in pH 7.73. MWCNTs modified CPE also catalyzes the oxidation of quercetin in presence of kaempferol and gives a different anodic peak which shows both can be determined simultaneously. Simultaneous determination of kaempferol and quercetin was carried out under similar conditions as that of kaempferol. The real sample analysis of kaempferol has been carried out in corms and petals of saffron (*C. sativus*) using standard addition method. The average percent recoveries of 99.84 and 99.26 in corms and petals of saffron, respectively shows a good validity of the proposed method. The accuracy and precision of the method are also determined and validated statistically. The developed SWV method can be used in quality control laboratories for rapid and accurate determinations of quantitative values of kaempferol and quercetin in phytomedicines, pharmaceutical preparations and in biomedical fluids like serum and urine. The developed method will also be useful for its application in pharmacokinetic, pharmacodynamic, bioavailability/bioequivalence studies.

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